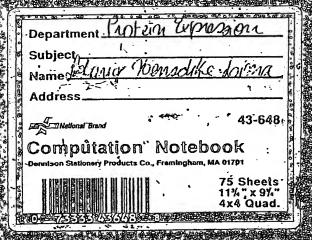
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Ruben EXHIBIT #70

Department Not an Expression Subject Address Computation" Notebook 75 Sheets 11%" x 9%" 4x4 Quad. Ruben EXHIBIT 2070

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	2000	ldleded stock of virus C5 injected 22399 i
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	2 1 0/1	PCAQUE PUNGISHON OF BACULOUINUS STOCK
	31.27.	V CHICO IS: V.V MI. TOS MOST ST. V.
		6. Sequentially remove supernatant medium from each plate with a pipette and replace
		1771 1 A Live of the virus. Include
Ì		
		E12 Mani Sigo ploto Nami 2.1.94 the plates back and forth every 5 min to insure even adsorption of the virus.
	<u></u>	the state of the s
		Agrees Gel completely in a 70°C water bath
		(~10 min) and maintain at 37°C.
	ļ	Protocol - from Cybro - Bevs Methods.
	 	The state of the s
		To prepare the Grace's plaquing overlay:
	<u> </u>	PLAQUE PRODUCTION 8 Place a boute of sterile, dejonized, distilled
		Moverials: water and an empty, sterile, 100-ml glass
		In addition to the inaccitats insect in October 1985 to 3
		Information, page 3, you will need the terminal house of Grace's Insect Medium, 2X. Mix by
	┞╸ ┼╸┼╸	Exponentially growing Sf9 cells (viability >90%). GIBCO BRL 4% Agarose Gel (or GIBCO BRL pipetting, and move the mixture to 37°C pipetting, and move the mixture to 37°C pipetting, and move the mixture to 37°C pipetting.
	 	+ 4% Agarose Gel with Bluo-gal) 0. Combine 25 ml of Grace's Insect Medium,
		GIRCO RRI. Sf-900 Medium, 1.3X or GIBCO 2X: 12.5 ml of the sterile water, and 12.5 ml
	1 1 _	BRL Grace's Insect Medium, 2X; HI-FBS: of the melted 4% Agarose Get in the 37°C ontainer to make the plaquing overlay.
		Soulable electic container (-4" x 8" x 8") Maintain at 17°C to 42°C. Note: This exam-
	 	ale provides for a final agarose gel concentra-
	 	system tion of the tine to the time concentration may be
	4-4-4-	water complement of the overlay solution.
		27-C incubator
		10°C water bath Continue with protocol:
		37°C water bath 10. Sequentially remove inoculum from each plate with a Pasteur pipette and add 4 ml of
	1-1-1-	Protocol: the desired alaquing overlay. Note: This
	++++	.l. 1 Premie >1 ml of each of the log serial dilu-
		tions of the virus to be titered or purified from monolayer and premature gelling of the 1 x 10 ⁻² through 1 x 10 ⁻⁴ . overlay solution.
	1 1 1	Prepare a 50-ml stock suspension at 11 Allow overlays to solidify (10 to 30 min) at
		5 x 10 ⁵ cells/ml using Sf9 cells from an room temperature.
\vdash	++ +-	exponentially growing culture at >95%. 12. Carefully place the plates in a sealed con-
 	 -	tainer with a damp cloth (to provide humid- ity) and incubate at 28°C ± 0.5°C for 4 to 6
 		- + call culture diches, swirling gently while days. Note: Wild-type virus produces highly
L	<u> </u>	dispensing to insure even dispersal of cells. refractile, near-white plaques to the naked :
		4 Allow cells to settle to bottom of plate for eye. Recombinant virus produces mitky-gray
1		I h. Note: In serum-containing media, plaques of slight contrast without staining or transport the plates gently because cells
Ŋ		f v v v v v v v v v v v v v v v v v v v
II_		5 Inspert the cultures on an inverted micro-
		scope to confirm a 50% confluency. Adjust
		density as necessary.
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